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INFLUENZA IN 1948-1949

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Epidemiology in Europe

Experience, particularly in the last decade, has brought conviction that the epidemiology of influenza needs to be studied on a worldwide basis. As a result of a meeting held during the Fourth International Congress of Microbiology in Copenhagen in July 1947, the World Health Organization set up a World Influenza Centre (WIC) in the laboratories of the National Institute for Medical Research at Hampstead, London, England.[a] During 1948, this centre established contacts with most countries in Europe and with many elsewhere in the world. These either designated regional laboratories to collaborate with the centre or appointed observers to keep the centre supplied with information. Free exchange of information was also arranged with World Health Organization headquarters in Geneva, and with an influenza information centre in Washington. A beginning was also made in receiving workers for a short training in diagnostic techniques used in the study of influenza. During 1948, Dr. P. von Magnus, of the Statens Seruminstitut, Copenhagen, prepared diagnostic antigens A and B for performing haemagglutination-inhibition and complement-fixation tests, and reference immune sera ; these were distributed to collaborating laboratories.

During the winter of 1948-1949 an influenza epidemic due to virus A spread across western Europe and gave the centre an opportunity of showing its possibilities. It also revealed its defects and gave indications as to the chief needs for future improvements. A preliminary account of the outbreak has been published in the *Epidemiological and Vital Statistics Report*.[19]

Available official data vary greatly from country to country. In some, influenza is notifiable, but the clinical diagnosis is so uncertain that the

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available figures have a purely relative value as indicators of the activity of the disease. In others, notifications of deaths due to influenzal pneumonia are available. We also had reports from observers giving estimates of the incidence of the disease and news of varying degrees of probability gathered from the lay press. Where a real epidemic of influenza is occurring, there is but little doubt about it ; and, from the sources of information referred to, we have been able to piece together a picture, which we believe to be a fairly true one, of what happened in Europe during the winter of 1948-1949.

Various reports agree in stating that the epidemic began in Sardinia, was evident a little later in Sicily, and soon after, on the mainland of Italy. Thence it went north to Switzerland and France, west to northern Spain, east to Austria, and reached the Netherlands through Belgium about the New Year. There were no reports from Germany until early February ; the epidemic probably reached Scandinavia, but was not very troublesome ; it also spread to Iceland by late January. It spread through the countries of central Europe to Turkey, where a severe epidemic was reported beginning in mid-January. Cases began to appear in England early in January, but the epidemic was slow in developing and was less extensive than on the Continent. Virus was sent to the WIC laboratory from workers in France, Great Britain, Iceland, Italy, the Netherlands, and Switzerland ; and strains isolated from the epidemic were identical in type, as described below ; we will refer to this provisionally as 1949 A-prime. Some viruses from unconnected outbreaks were sent also from Czechoslovakia, Hungary, Sweden, and, one of obscure origin, from Turkey. Details of outbreaks in various countries will now be described in more detail.

Italy

Magrassi [12] describes the simultaneous occurrence of virus outbreaks in an area in northern Sardinia between mid-September and mid-October 1948, the peak incidence being 30-40 days after its beginning. Near the end of October, a number of small outbreaks appeared in a second area in northern Sardinia. There is a suggestion that these may have been, like those of the first batch, " autochthonous ", for they occurred almost simultaneously in separate localities in a restricted area. Shepherds living solitarily in open, deserted country are reported as being affected simultaneously with people in the nearest inhabited centre.

The officially recorded occurrences of influenza (records sent by the Istituto Superiore di Sanità in Rome) reached noteworthy proportions in Sardinia in November, with some indications of a rise in October. Also in Sicily and Calabria, November saw a rise. In the province of Trapani in Sicily, a small outbreak of 43 cases was actually recorded in September.

Over the rest of Italy, cases were reported in numbers by December, and the peak was reached in January. The numbers of actually notified cases were hundreds of times lower than those estimated by observers ; more than 50% incidence was recorded for some areas in Sardinia by Magrassi. Schools were closed on the mainland in mid-December ; deaths were reported amongst the very old and the very young.

Earlier reports suggested that influenza virus B was concerned ; though it may have caused some local outbreaks, it seems likely that the main causative agent was an A virus of the 1949 type which can be included in the A-prime group (see above).

Consideration will be given later to an outbreak of influenza in southern Italy, Sardinia, and Sicily, in the late spring of 1948 and to its possible bearing on the autumn-winter epidemic.

Switzerland

Notified cases of influenza in January 1949 were ten times higher than those in December 1948. Doubtless the outbreak began in December, as the peak was reached in the week of 30 January to 5 February. The cantons on the frontiers, Tessin, Bâle, Genève, and the Grisons, were attacked first and most heavily. Serological studies in Dr. G. Weisflog's laboratories in Berne revealed that virus A was concerned ; a single case of virus B infection was detected serologically. Dr. J. Wirth, in Professor E. Grasset's laboratory in Geneva, isolated a strain of virus A which proved to be of the 1949 A-prime type.

Austria

Little information is available apart from reports of occurrence of several thousand mild cases at the beginning of January 1949 in the Tyrol ; the outbreak later reached Vienna.

France

Influenza spread into southern France from Italy during December 1948, appearing first in the region of Haute-Savoie. It extended rapidly to the north and east and to the Paris region. Several reports stated that 20% of the population were attacked. In the laboratories of Dr. Dujarric de la Rivière and Dr. P. Lépine at the Institut Pasteur, viruses were isolated at about the New Year and proved to be of the 1949 A-prime type. Early in January Dr. J. A. Dudgeon visited Paris on behalf of the World Influenza Centre and brought back information and specimens of throat-washings. By early February the epidemic was declining, but there was a relatively larger number of severe cases. Subsequently Dr. Lépine and his collaborators [11] reported the isolation of ten strains, of which some correspond to the 1949 A-prime (FM in their paper), and others to typical A

(PR8). These strains are still under study ; their relationships to the European epidemic as a whole are obscure ; they will be discussed in a subsequent paper.

Belgium

Unofficially, reports suggested that there was a widespread epidemic of an influenza-like disease which spread from France through Belgium to the Netherlands. Officially, however, no information was forthcoming of the occurrence of anything other than a " mild disease like the common cold, as seen annually ".

Netherlands

The influenza epidemic apparently reached the southern part of the Netherlands at the beginning of January, spreading from France through Belgium. Professor J. Mulder, of Leiden, was the first of the observers on the Continent to send us full information and strains isolated by himself. Information came also from Professor J. D. Verlinde of the Instituut voor Praeventieve Geneeskunde, Leiden. The figures we received suggested that influenza finally attacked the whole country fairly uniformly, with an incidence of 5%-7% of the population. Incidence was equally high in town and country districts. The peak of mortality was reached in the second week of February. Just under 2,000 deaths were recorded for the months of January and February, a figure higher than for any epidemic in the Netherlands since 1941. While most of the cases were mild, some fulminating cases of tracheo-laryngo-bronchitis occurred, associated with infection with staphylococci. Serological evidence indicated the occurrence also of a little sporadic B infection.

West Germany

Reports from the US zone of occupation, Heidelberg, reported serological evidence of the presence of A infection from 16 January 1949. On 9 February it was reported that mild influenza was scattered over much of Germany. The peak incidence of notified cases occurred in February in the US and French zones, and in March in the British zone.

United Kingdom

The first cases were recognized in England at the very beginning of January, some of them in people just arrived from Paris ; A-prime virus was isolated. Nevertheless, an epidemic was very slow in developing and never reached great dimensions. In London, the peak of deaths recorded as due to influenzal pneumonia was not reached until the end of February, and in most of the country not till the middle of March. The figure for the highest number, per week, of such deaths in the 126 great towns was 379 ; in years of serious epidemics, the figure of 1,000 has been

exceeded. One or two local outbreaks were apparently due to virus B but, in general, all the evidence pointed to the A-prime virus as the causative agent. Viruses were isolated at the Public Health Laboratories in London (Colindale, Dr. F. O. MacCullum) and Northampton (Dr. L. Hoyle).

In Scotland, peak incidence was at the end of February ; in general, influenza was apparently less prevalent than further south. A-prime virus was isolated by Dr. R. H. Swain in Edinburgh.

Ireland

Ireland had no epidemic sufficient to increase the notified deaths from influenzal pneumonia appreciably above the normal for the first quarter of the year. Small groups of cases were notified from scattered centres, but there is no laboratory confirmation that influenza virus A or B was concerned. During May, Dr. P. Meenan, of the Dublin Department of Health, found serological evidence of A infection in one of three pairs of sera submitted to him from Dublin. This affords the only evidence that virus A definitely reached the country.

Denmark

Influenza was prevalent in Denmark from January onwards, but the epidemic was apparently not as extensive as in other parts of Continental Europe. The available serological evidence and the shape of the incidence curve together suggest that a B outbreak was succeeded and partly overlapped by an A outbreak (information from Dr. P. von Magnus). The peak incidence in the provinces of Aalborg and Randers occurred in January ; there is no evidence as to whether A or B was concerned. In Copenhagen the peak was reached in February, and B was almost certainly to blame then, for complement-fixation tests done in that month showed high values against B but not against A. On the other hand, similar tests in March indicated that virus A was predominantly concerned. Bornholm had rather a high peak in February, but most other centres had maximal incidence in March. We may suspect virus A, but laboratory evidence is available only from Copenhagen. No virus strains were isolated, so one cannot be certain that the 1949 A-prime virus was concerned.

Sweden

Sweden suffered from some influenza due to virus B. Dr. A. Svedmyr, at Stockholm, told us of small local outbreaks at Stockholm early in February 1949. Dr. G. Löfström, from Uppsala, reported that influenza was passing slowly through the country with no sharp peak in the curve of incidence ; the curve showed a flat plateau throughout February and March, never reaching the level of a real epidemic. In one local epidemic at a military camp at Gothenburg, virus A was apparently concerned.

Iceland

First cases were reported during January 1949 in personnel and travellers at Keflavik, an airport near Reykjavik with much international traffic ; cases were also reported in the Thingeyri region in the extreme north-west, following a visit from a trawler coming from abroad. The main outbreak, however, really began in late January, affected the whole island, and was unusually prolonged. The peak incidence occurred in February in two districts in the north-west ; some areas in the south and south-west reached their peak in March, but the incidence was greatest for the whole island in April, and the epidemic dragged on locally even till July and August. In contrast, an epidemic in 1946 lasted only five weeks. Altogether about 9,000 cases were notified up to September 1949, but these " undoubtedly only represent a fraction of the total cases ". Four strains of virus were isolated by Dr. B. Sigurdsson, of the Institute for Experimental Pathology at Reykjavik, and examined at the centre ; they were of the A-prime (1949) type (A/Reykjavik 1, 2, 3 and 4/1949). The results of studies of human sera were, however, difficult to interpret.

Czechoslovakia

A B-outbreak apparently began in Slovakia in October 1948, spread all over that province, and was maximal in January 1949. Dr. D. Blaškovič, in Bratislava, sent us a strain of B which showed serological differences from the classical Lee strain. Late in February 1949 a small number of patients and sera showed a rise in antibody against A, but there was no noteworthy outbreak. Influenza is not notifiable in Bohemia and Moravia, but reports indicate that the disease was less prevalent there than in Slovakia.

Hungary

In Hungary, where complicated influenza is reportable, there was no striking rise in incidence, which remained well below the 1931-1937 median. Indeed, Hungary has apparently been free from serious trouble from influenza for the last decade. Such influenza as there was in 1949 occurred during March, when Dr. E. Farkas, of the State Institute of Hygiene, Budapest, isolated three strains of influenza B virus from sporadic cases in Budapest. He also obtained one strain from a local epidemic in the village of Valkó, 20 miles east of Budapest ; here, about 300 cases occurred amongst 1,900 inhabitants. The Valkó virus proved to be an A-prime.

Yugoslavia

Yugoslavia appears to have had a widespread epidemic of an upper respiratory infection ; the nature of this is, however, from both clinical and serological data, doubtful. The peak was in the latter half of March ;

Dr. A. Terzin, of the Federal Institute for the Study of Epidemics, Belgrade, estimates that 10% of the population were affected ; but only 1% came under medical control, and of this 1%, only 1-2% showed severe clinical symptoms. An account by Dr. I. Kankler, of Ljutomer, Slovenia, speaks of a prodromal period of about two days, and an occurrence " in most cases " of pharyngitis, laryngitis, and tracheobronchitis, as well as gastro-intestinal disturbance and much sweating during convalescence. This description differs in several respects from the picture of the epidemic seen in western Europe. In conformity with this, Dr. Terzin's serological studies on cases in Belgrade, confirmed in six instances by us, show no evidence of A or B infection, except for single instances with antibody rises against B and, doubtfully, A.

Turkey

A considerable epidemic broke out in Ankara, beginning in mid-January 1949 and reaching its peak during the first three weeks of February. It suddenly subsided during the fourth week. In different parts of Ankara the morbidity was from 25% to 80%. Closing of schools became necessary, the incidence in schoolchildren being about 40%. Later, the epidemic spread to Istanbul and other parts of Turkey. In Ankara there was, during the first three weeks of February, a rise in " bronchopneumonia " deaths to 32, 53, 46 respectively ; corresponding figures for 1948 were 17, 14, 14. Four cases of encephalitis occurred during the epidemic, and complement-fixation tests against influenza A were positive in serum dilutions of 1 : 32 ; it is possible, therefore, that these were complicated cases of influenza. Dr. S. Payzin, of the Refik Saydam Institute of Hygiene at Ankara, carried out complement-fixation tests which showed clearly that the epidemic was due to a strain of influenza virus A. He also isolated some strains of virus, but the relation of these to the outbreak is not certain. We cannot say, therefore, whether the outbreak in Turkey was due to the same serological type of virus as affected western Europe.

Other European countries

Official statistics are available from the *Epidemiological and Vital Statistics Report* [19] concerning some countries with which the World Influenza Centre had no direct contacts. It appears from these that influenza in excess of normal occurred in Spain (maximum in February), Finland (maximum in March), Norway (maximum in January), and Poland. In the last country, notified cases in March 1949 were 100 times as many as in 1948, and it is indeed unfortunate that no information concerning laboratory studies is available from there. Roumania, Greece, and Portugal seem to have escaped, so far as the official records reveal.

Antigenic Studies of Virus Strains

The first two strains of influenza virus (A/Nederland-1/49, A/Nederland-2/49) received at the World Influenza Centre (WIC) from the 1948-1949 European epidemic were isolated by Professor Mulder in Leiden, Netherlands, at the beginning of January 1949. Haemagglutination-inhibition tests with stock antisera soon established that they were closely related to the A-prime viruses, Barratt (England, 1947), FM1 (USA, 1947), and not closely related to PR8. On 17 January, the strain A/London-1/49 was isolated from the lung suspension of a fatal case of staphylococcus pneumonia in London. At that time an epidemic was widespread in Paris and, on 19 January, the strain A/Paris P.L. 1/49 was isolated from a throat-washing collected by Dr. Dudgeon. Both the London and the Paris strains were again found to be antigenically closely related to A-prime viruses and not to PR8. These early findings, together with the results of examination of human sera from Paris and Geneva, enabled us to form the opinion that the epidemic then prevalent in Europe was most likely due to a virus closely similar to the A-prime group of viruses which had caused widespread epidemics in 1946-1947. Subsequently, virus strains were received from various countries in Europe as well as from Canada, USA (including Puerto Rico), and Ocean Island, in the South Pacific. Altogether, 86 strains of influenza A and 10 strains of influenza B were added to our collection (see Annexes 1 and 2). Twenty representative A strains were chosen for more detailed studies. The correct typing of nine B strains was confirmed, but as yet no detailed antigenic comparisons have been made. The results of these investigations are reported in this paper. Studies on other A strains from Turkey, France, Canadian Arctic, and one each from Germany and Italy, owing to their peculiar characters, will be dealt with in a subsequent paper.

Designation of strains

Since more and more influenza strains are being isolated and given arbitrary designations bearing no reference to the epidemiological circumstances of their occurrence, it becomes increasingly difficult for individual workers to discover the circumstances of their origins. In 1948, a letter from WIC containing suggestions regarding future designation of influenza strains was circulated to many interested laboratories. This letter suggested describing the serological type, the year, and the place of origin thus : A/Nederland-1/49, A/Nederland-2/49 for the first two Dutch A strains isolated in 1949. Such an abbreviation as Ned. 1 is convenient when a strain is mentioned a second time in a communication. Some laboratories have agreed with these suggestions and have designated their strains accordingly. Others have continued to send in strains under more or less arbitrary designations. Since, in many cases, the original workers

have already published facts about their strains under such designations, it is thought that, for the time being, less confusion will arise by retaining these. Consequently, in this article, many strains appear under the WIC designation, while others bear arbitrary designations followed by a bracket to indicate the place and year of isolation.

Laboratory variation of influenza virus

It is now generally recognized that influenza virus may vary in antigenic and other properties under laboratory conditions. Variation in the antigenic make-up of two different substrains of PR8 has been reported by Francis.[4] The conditions determining such variation are still obscure. Hirst [7] first reported antigenic variations in influenza A virus after mouse adaptation. This type of variation appears to occur frequently, though not regularly (Sugg [21]). Most previous workers have demonstrated antigenic variations by the haemagglutination-inhibition and neutralization techniques. Using a strain-specific complement-fixation test in addition to the haemagglutination-inhibition test, we have studied this problem with a number of influenza strains; the results may be summarized here. Two instances of spontaneous antigenic variation have been encountered, in the one case involving a relatively small difference between two substrains of PR8, in the other case involving a wide difference between two substrains of WS. Mouse adaptation of two strains of A-prime virus has produced small but definite antigenic deviation from their non-adapted parent strains. No significant antigenic variation was observed in two freshly isolated viruses after 12 passages, under standard conditions, in the allantoic sac of chick embryos. This, however, does not exclude the possibility of antigenic changes in virus cultivated on eggs, as these may occur after more prolonged passage or cultivation under certain conditions. These laboratory changes are extremely important in any antigenic studies of influenza virus ; their bearing on the results of the present studies will be discussed later.

General characters of influenza A strains isolated in 1948-1949

Strains of influenza A isolated in 1948-1949 exhibit certain general characters which may be summarized as follows :

(1) With our technique of isolation, i.e., intra-amniotic inoculation into 13-day-old chick embryos followed by incubation at 35° C for four days, all four strains of virus isolated in this laboratory (Paris-1, London-1, London-4, and an unnamed strain which was subsequently discarded) gave a positive haemagglutination test on the first egg passage ; the infected amniotic fluid agglutinated fowl, guinea-pig, and human red cells. Similar results were obtained by Professor C. H. Stuart-Harris (personal communication) who made a larger number of isolations.

(2) All four strains isolated by us grew readily in the allantoic sac in their second passage, producing a fowl haemagglutination titre of about 1/160-1/320. Two strains (Paris-1, London-1), whose multiplication on allantoic passage has been followed, showed a rise in titre (1/640) after 5-6 passages, and a further rise (1/1280-1/2560) after 9-12 passages. In later passages their maximal haemagglutination titre remained more or less steady.

(3) Of 20 strains studied in ferrets, most gave a moderate temperature response (104° F (40°C). The ranges of the antibody titres obtained in the ferret sera against homologous viruses as determined by haemagglutination-inhibition test were : 1/40-1/80 in 4 strains ; 1/160-1/320 in 12 strains ; and 1/640-1/1280 in 4 strains. Thus, in general, these viruses appear to be moderately virulent for ferrets and stimulate a moderate amount of antibody response.

(4) None of 11 A strains studied was initially virulent to mice, i.e., they did not cause death nor any significant lung lesions. Two strains (Paris-1 and Roma-2) began to cause death and more or less extensive lesions after six mouse passages. However, allantoic-fluid virus from Paris-1 after 24 mouse passages and from Roma-2 after 14 mouse passages had a mouse titre (50% maximal score lesions) of only 10^{-4} and 10^{-3} respectively. The titration curves were very much flattened so that these titres can be considered only approximate. Only very low haemagglutination titres (1/10-1/40) could be obtained with the tracheal washings of mice infected with mouse-adapted Paris-1 or Roma-2 (Panthier et al.[15]), in spite of the complete pulmonary consolidation. Under the same circumstances, we had no difficulty in obtaining from tracheal washings infected with PR8 a haemagglutination titre of 1/5120.

(5) The haemagglutination was inhibited to moderate titres (1/80-1/160) in nine strains studied by unheated normal ferret and mouse sera.

(6) In heamagglutination titrations at room temperature, agglutination of red cells near the end-point tended to weaken rapidly on standing, so that the titres were usually one tube lower when read after one hour as compared with readings taken after 30 minutes ; this phenomenon is particularly marked with fluids from the first few egg passages. Although no detailed studies were made on the adsorption-elution curves of these viruses, experiences gained in the preparation of eluate antigens indicated that they eluted more readily and completely from fowl red cells at 37° C than do either PR8 or WS.

(7) Of over 20 strains examined by electron-microscopy after adsorption on red cell " ghosts ", all showed a large proportion of long filamentous forms. Such forms were also found in recently isolated 1943 A and 1947 A-prime viruses, but were on the whole shorter and less numerous. (Chu, Dawson & Elford. [3])

Technique of haemagglutination-inhibition test

The technique of the haemagglutination-inhibition test is well known. In recent years, however, conflicting opinions have arisen regarding the value of this test in the study of freshly isolated viruses ; for this reason, we wish to describe our technique in some detail.

Sera. Sera were obtained from ferrets bled 12-16 days after intranasal infection and usually from a single animal for each strain of virus. Animals were kept under strict isolation precautions. All sera were stored at −60° C.

Sera were treated in either of two ways as indicated in each experiment :

(1) Heated at 56° C for 30 minutes immediately before the test.

(2) Treated with an equal volume of crude cholera filtrate at 37° C for 18-22 hours followed by heating at 56° C for one hour. This method was first described by Mulder & van der Veen. [13] The cholera filtrate was prepared by growing Z-4 strain of *V. cholerae* in soft agar medium (Burnet & Stone [2]) for 16 hours and filtering the expressed juice through a Seitz E. K. pad. Purified receptor-destroying enzyme (RDE) should not be used. We have confirmed the findings of Mulder et al. [14] that thorough pre-treatment of ferret sera with crude cholera filtrate followed by heating at 56° C for one hour invariably destroyed the non-specific inhibitory power of normal sera to undetectable level, whereas the antibody titre of immune sera was not significantly altered. However, cholera receptor-destroying enzyme purified by adsorption and elution from fowl red cells and having ten times the RDE potency of crude filtrate was entirely ineffective in destroying the normal ferret serum inhibitor, even in the presence of added Ca^{++}. Furthermore, the supernatant fluid of the crude filtrate after removal of RDE activity was fully active. The mechanism of action of cholera filtrate on the non-specific inhibitor therefore is still obscure.

Antigens. In most cases, red cell eluate antigens were used ; in a few cases, however, freshly harvested allantoic fluids have been used instead. As far as possible, viruses with the minimal number of laboratory passages were used, both for infecting ferrets and for antigen preparation. Eluate antigens were preserved with 0.05% sodium azide and stored at 4° C ; under such conditions they remained antigenically stable for at least ten months.

Red cells. Four fowls were selected for the uniform behaviour of their red cells in haemagglutination and inhibition tests. They were bled in turn. The citrated blood was kept at 4° C and used within one week. A 0.5% suspension was prepared each day from freshly washed red cells and standardized in a photo-electric absorptiometer.

The test. A modified Salk's pattern method [17] was used. To 0.25 ml of twofold dilutions of serum, 0.25 ml of red-cell suspension was added, followed at once by 0.25 ml of diluted antigen containing eight partial-agglutinating units of virus. After standing at room temperature (20°-25° C) for one hour, the highest serum dilution which completely inhibited agglutination was read as the titre. All titres referred to in this article are expressed in terms of the initial dilutions.

Set-up of experiments. When the antigenic relationships between several strains of virus were to be determined, these viruses and their respective sera were always crossed in the same experiment. All sera were diluted in bulk and then distributed in 0.25-ml quantities. When these precautions are taken, an intrapolation of serum titre between two dilutions is justified, and the effects of minor variations in experimental conditions, such as temperature, red cells, and strength of antigens used, are automatically eliminated.

Analysis of results. Hirst [6] pointed out that different preparations of the same strain of virus may, with the same serum, give different titres which are, in all probability, unrelated to genuine antigenic differences ; to correct this apparent irregularity in reactivity, Hirst introduced the avidity factor. On a similar basis we have employed a different method of calculation to obtain what we have termed the " antigenic ratio ".

Suppose the antigenic relationship between two viruses, I and II, is to be determined from the following experimental data :

Serum	*Antigen*		*Ratio :* $\frac{\textit{Heterologous titre}}{\textit{Homologous titre}}$
	Virus I	*Virus II*	
Anti-I	640	1280	$\frac{1280}{640} = \frac{2}{1}$
Anti-II	80	2560	$\frac{80}{2560} = \frac{1}{32}$

then, Antigenic ratio $= \sqrt{\frac{2}{1} \times \frac{1}{32}} = \sqrt{\frac{1}{16}} = \frac{1}{4}$

This antigenic ratio ¼ implies that, if the reactivity of the two virus antigens were the same, anti-I serum should react with virus II to ¼ of its homologous titre and vice versa. This implication may not be true, but, in the absence of more precise knowledge concerning the antigenic composition of influenza virus, the antigenic ratio does seem to be a convenient expresssion of antigenic relationship between two strains, provided the inherent limitations of its significance are fully appreciated. Thus, with antigenically widely divergent strains, the ratio heterologous/homologous titre may vary to a certain extent in different ferrets and in the same ferret when bled at different times following infection ; such

antigenic ratios as $^1/_{16}$ or $^1/_{32}$ merely indicate a fairly wide antigenic difference between the strains concerned, but do not necessarily mean a greater difference in the latter case than in the former. On the other hand, ratios within the range $^1/_1$ to $^1/_{1.5}$ would mean that the strains concerned are antigenically indistinguishable ; this is of greater significance since, in our experiences of such cases, sera from different ferrets or those bled at different times gave practically constant antigenic ratios. A numerator exceeding one such as $^{1.5}/_1$ would mean a bigger heterologous than homologous reaction ; this rarely happened and is likely to be due to technical inaccuracies.

Antigenic relationship of 1948-1949 A Viruses to WS, PR8, FM1, and A/Paris P.L. 1/49

The antigenic ratios between 20 strains of 1948-1949 A virus and WS, PR8, FM1, and A/Paris P.L. 1/49 are shown in table I. The origins of the four standard strains are as follows :

WS/38	A recent egg passage of mouse-lung virus dried in 1938.
PR8/R	Rockefeller Institute strain of PR8, sent to us as dried allantoic fluid in 1943.
FM1	Originated from dried allantoic fluid sent to us from Dr. Löfström, Sweden.
A/Paris P.L. 1/49	Isolated in 1949 ; had one amniotic and two allantoic passages.

The 20 strains listed in table I are all closely related to FM1 and to Paris-1, indicating that they all belong to the A-prime group. None of the 20 is related to WS or PR8. Furthermore, of the 20 strains, all but three (Ocean Island, Nederland-3, and MUL) are distinctly more closely related to Paris-1 than to FM1. In fact, with most of these strains any small differences from Paris-1 can well be within the limits of experimental error. Such minute differences as $^1/_{1.2}$ are not technically reproducible and therefore not individually significant. But taking many strains into consideration, we are inclined to believe that even these minute differences may be statistically significant since, if they were entirely due to random experimental errors, one would expect to get as frequently ratios of more than unity such as $^{1.2}/_1$; this did not happen. The strain Ocean Island 1 which was derived from an isolated outbreak (Isaacs et al. [9]) appears to belong to the A-prime group but is less closely related to Paris-1.

Antigenic relationship of 1948-1949 A viruses to each other

Numerous tests have been performed to determine the antigenic relationship to each other of strains within the group of 1948-1949 viruses ; only the results of a few illustrative experiments will be presented.

TABLE I. ANTIGENIC RATIOS BETWEEN STRAINS OF INFLUENZA A VIRUS ISOLATED IN 1948-1949 AND SOME SELECTED STRAINS

1948-1949 Strains	WS	PR8	FM1	A/Paris P.L. 1/49
Ed-1 (Edmonton, Canada, 1949)	$< \frac{1}{28}$	$< \frac{1}{96}$	$\frac{1}{3}$	$\frac{1}{1.15}$
A/Paris P.L. 1/49	$< \frac{1}{18}$	$< \frac{1\,a}{28}$	$\frac{1\,a}{2.4}$	$\frac{1}{1}$
D48 (Paris, 1948)	$< \frac{1}{28}$	$< \frac{1}{96}$	$\frac{1}{3.5}$	$\frac{1}{1}$
A/Nederland-1/49	$< \frac{1}{36}$	$< \frac{1}{124}$	$\frac{1}{3.5}$	$\frac{1\,a}{1}$
A/Nederland-2/49	$< \frac{1}{32}$	$< \frac{1}{111}$	$\frac{1}{3.5}$	$\frac{1}{1.2}$
A/Nederland-3/49	$< \frac{1}{20}$	$< \frac{1}{68}$	$\frac{1}{2.3}$	$\frac{1}{2}$
A/Nederland-4/49	$< \frac{1}{28}$	$< \frac{1}{96}$	$\frac{1}{3.1}$	$\frac{1}{1.3}$
A/Nederland-5/49	$< \frac{1}{28}$	$< \frac{1}{85}$	$\frac{1}{1.9}$	$\frac{1}{1.3}$
A/Nederland-6/49	$< \frac{1}{37}$	$< \frac{1}{112}$	$\frac{1}{3.5}$	$\frac{1}{1.6}$
A/Budapest-4/49	$< \frac{1}{15}$	$< \frac{1}{52}$	$\frac{1}{1.5}$	$\frac{1\,a}{1}$
A/Reykjavik-2/49	$< \frac{1}{21}$	$< \frac{1}{73}$	$\frac{1}{2.5}$	$\frac{1\,a}{1.2}$
A/Roma-2/49	$< \frac{1}{37}$	$< \frac{1}{112}$	$\frac{1}{2.2}$	$\frac{1}{1}$
MUL (Italy, 1949)	not tested	$< \frac{1}{17}$	$\frac{1}{2.8}$	$\frac{1}{2}$
A/Ocean Island-1/48	$< \frac{1}{34}$	$< \frac{1}{104}$	$\frac{1}{2.83}$	$\frac{1\,a}{5.1}$
A/Geneva-1/49	$< \frac{1}{52}$	$< \frac{1}{129}$	$\frac{1}{2.5}$	$\frac{1}{1.1}$
A/London-1/49	$< \frac{1}{39}$	$< \frac{1}{120}$	$\frac{1}{2.7}$	$\frac{1\,a}{1}$
A/London-3/49	$< \frac{1}{68}$	$< \frac{1}{206}$	$\frac{1}{2.5}$	$\frac{1}{1.4}$
Ashmore (Catterick, England, 1949)	$< \frac{1}{13}$	$< \frac{1}{44}$	$\frac{1}{2.7}$	$\frac{1\,a}{1}$
Knowles (Sheffield, England, 1949)	$< \frac{1}{15}$	$< \frac{1}{52}$	$\frac{1}{3.1}$	$\frac{1\,a}{1}$
Coamo-1 (Puerto Rico, 1948)	$< \frac{1}{55}$	$< \frac{1}{192}$	$\frac{1}{2.5}$	$\frac{1}{1.15}$

a Sera simply heated at 56° C for 30 minutes, not treated with cholera filtrate

At the outset, we were impressed by the finding that the 1948-1949 viruses appeared to be antigenically more homogeneous than any other groups encountered previously. We had the pleasure of discussing our results with Professor Mulder, who said that somewhat different results were obtained from the collection of Nederland strains which he had studied. Besides the difference in strains under study, another difference arose from the fact that Mulder employed, as a routine, ferret sera from which the non-specific inhibitor was removed by treatment with cholera filtrate. Consequently, we repeated our earlier experiments, using in parallel simply heated sera and sera treated with cholera filtrate. The results obtained are shown in table II. Similarly, we have since studied the same Nederland strains investigated by Mulder. The results are shown in table III. These data demonstrate that, within the limits of technical error, essentially similar results were obtained whether the sera used were simply heated or treated with cholera filtrate. A slightly bigger difference did exist amongst the four Nederland strains than amongst the others, but

TABLE II. ANTIGENIC RATIOS BETWEEN 1948-1949 STRAINS OF INFLUENZA A VIRUS BY TWO TECHNIQUES

	Technique [a]	D48	Geneva-1	Paris-1	London-3	Roma-2
D48 (Paris, 1948)	A	1/1	1/1.3	1/1.3	1/1.5	1/1.6
	B	1/1	1/1.2	1/1	1/1.9	1/1.4
Geneva-1	A		1/1	1/1.2	1/1	1/1
	B		1/1	1/1	1/1	1.1/1
Paris-1	A			1/1	1/1.15	1/1.25
	B			1/1	1/1.4	1/1
London-3	A				1/1	1/1.15
	B				1/1	1/1
Roma-2	A					1/1
	B					1/1

[a] Technique A : Sera heated at 56° C for 30 minutes
Technique B : Sera treated with cholera filtrate at 37° C for 18-22 hours before heating at 56° C for one hour

TABLE III. ANTIGENIC RATIOS BETWEEN 1948-1949 STRAINS OF INFLUENZA A VIRUS BY TWO TECHNIQUES

	Technique[a]	Paris-1	Nederland-3	Nederland-4	Nederland-5	Nederland-6
Paris-1	A	1/1	1/2	1/1.2	1/1.2	
	B	1/1	1/2	1/1.3	1/1.3	1/1.6
Nederland-3	A		1/1	1/1.6	1/1.6	
	B		1/1	1/1.4	1/1.8	
Nederland-4	A			1/1	1/1.4	
	B			1/1	1/2.4	
Nederland-5	A				1/1	
	B				1/1	
Nederland-6	A					1/1
	B					1/1

[a] Technique A : Sera heated at 56° C for 30 minutes
Technique B : Sera treated with cholera filtrate at 37° C for 18-22 hours before heating at 56° C for one hour

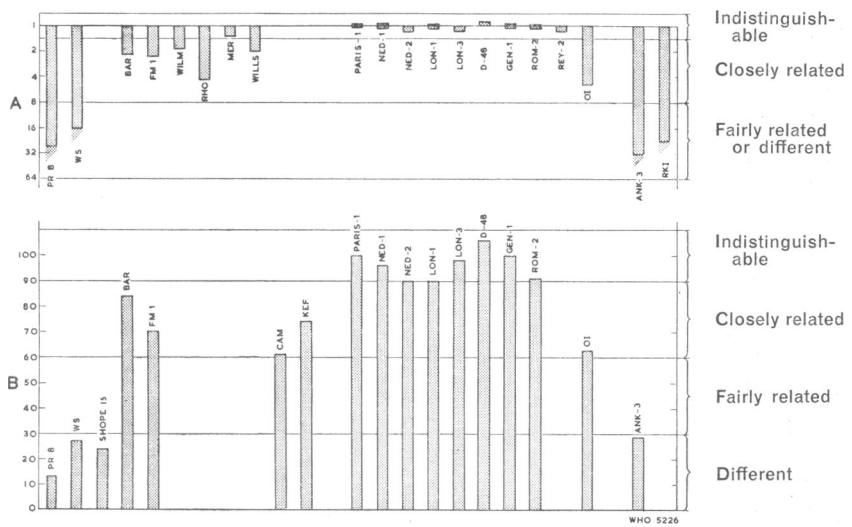
these differences are small in comparison with those existing among different strains of 1946-1947 A-prime virus. It may also be noted that the four Nederland strains had had many more egg passages than the other 1948-1949 viruses which we have studied (see Annex 1, page 24).

Our conclusion as to the comparative merits of the two techniques (heating or treatment with cholera filtrate) is that in the study of closely related strains, when fairly high inhibition titres are dealt with in any case, pretreatment of sera with cholera filtrate does not materially alter the experimental results. (Recently, we have used ferrets bought from dealers and have found much more variable and sometimes much higher non-specific inhibitor levels in their sera, even after heat inactivation, than hitherto encountered in our inbred stock. In such cases, removal of non-specific inhibitor becomes essential.) However, when rather low titre sera are being used, or when distantly related strains are being studied, cholera filtrate treatment does offer an advantage in telling whether a low titre inhibition is due to genuine antibody reaction or merely to non-specific inhibitor.

Relationship of 1948-1949 strains to 1946-1947 strains

The above experiments suggest that the majority of 1948-1949 viruses form a rather homogeneous group, but differ a little from the FM1 strain. The FM1 virus employed in our studies has had a long history of laboratory passage in the course of which slight deviation from its original antigenic pattern may conceivably have occurred. In order to ascertain the true relationship between viruses from these two years, comparison with other 1946-1947 strains is clearly necessary. Unfortunately, the position is complicated by the fact that the 1946-1947 viruses are rather heterogeneous amongst themselves ; certain strains such as FM1 (USA), Barratt (England), Williams (England), and G-g (Sweden) fall into a reasonably homogeneous group by both inhibition and strain-specific complement-fixation test, whereas others, such as Cam (Australia), Keffer (USA), Rhodes (USA), and Leiden (Netherlands), appear to have individual characteristics by one or both tests. We failed to identify Paris-1 with any of these strains, but, on the whole, it is more closely related to the Barratt and Williams than to any others. The average antigenic ratio from four tests is $^1/_{2\cdot3}$ and $^1/_{1\cdot9}$ respectively (fig. 1). Finally we compared three freshly isolated strains from each of the two years with each other and with Barratt

FIG. 1. ANTIGENIC RELATIONSHIP OF STRAIN PARIS-1 OF INFLUENZA A VIRUS WITH 22 OTHER STRAINS



(A) shows the reciprocals of antigenic ratios obtained by the haemagglutination-inhibition test.

(B) shows the percentage relationship obtained by the strain-specific complement-fixation test of Fulton & Dumbell.[5]

The divisions on the right are empirical and give approximate indications, for the respective tests, of zones within which the strain concerned might be considered indistinguishable from, closely related to, fairly related to, or different from Paris-1.

TABLE IV. ANTIGENIC RATIOS BETWEEN 1946-1947 AND 1948-1949 STRAINS OF INFLUENZA A-PRIME VIRUS

	Barratt	Wills	Mercer	Williams	Neder-land-1	Geneva-1	Roma-2
Barratt (England, 1947)	1	1/4.9	1/2.8	1/1.2	1/1.6	1/1.3	1/1.26
Wills (England, 1947) AM3AL2		1	1/1.22	1/4.9	1/2.6	1/2.4	1/2
Mercer (England, 1947) AM1AL3			1	1/3.1	1/1.5	1/1.6	1/1.5
Williams (England, 1947) AM1AL2				1	1/2.6	1/2	1/2.4
Nederland-1 (1949)					1	1/1	1.12/1
Geneva-1 (1949)						1	1/1
Roma-2 (1949)							1

All sera treated with cholera filtrate

(table IV). Owing to lack of any clear-cut patterns, the results are rather difficult to interpret. In general, the 1948-1949 viruses bear a close relationship with all four of the 1946-1947 strains tested ; with two of them (Barratt and Mercer), the differences can hardly be considered significant. On the other hand, one will note that Barratt and Williams significantly differ from Mercer and Wills, even though all four were isolated in England during the same epidemic and, with the exception of Barratt, had only three to five egg passages.

In conclusion, therefore, we feel that the 1948-1949 viruses are also remarkably close to some strains of 1946-1947 A-prime viruses, so that one cannot with certainty differentiate strains of 1949 from all those of the earlier years.

Strain-specific, complement-fixation test

The technique of Fulton & Dumbell [5] was adopted with the following modifications :

(1) Eluate antigens were preserved with sodium azide and stored at 4° C.

(2) Twelve instead of 16 complement dilutions as described in the original test were found to be adequate and greatly simplified the technical performance. Antisera were prepared from a pool of 40 convalescent mice.

Fulton & Dumbell have shown that their test readily reveals antigenic similarity or differences between influenza virus strains, a fact which we

have confirmed. In general, the haemagglutination-inhibition and complement-fixation tests gave comparable results ; in certain cases, however, the latter appears to be less sensitive than the former. For example, three strains of 1947 A-prime virus, Barratt (England), Leiden (Netherlands), and Rhodes (USA) were found to be antigenically identical by the complement-fixation test, yet definite differences were detected by the haemagglutination-inhibition test. This discrepancy may be due to the fact that, in the complement-fixation test, pooled sera from at least 40 mice were used whereas, in the inhibition test, sera were prepared from a small number of ferrets (usually only one). It is still premature to give an opinion on the relative merits of the two tests, but we believe that the complement-fixation test is a useful check on results obtained with the haemagglutination-inhibition test, particularly when freshly isolated strains susceptible to non-specific inhibitor are being studied. This belief is based on the actual results which we have obtained with the 1948-1949 strains, as shown in fig. 1. It can be seen that, of all the strains studied in relation to Paris-1, three (PR8, WS, and Shope) are widely different ; the 1946-1947 viruses, Barratt (England), FM1 (USA), Cam (Australia), and Keffer (USA) are closely related ; so is Ocean Island 1 ; and seven strains of 1948-1949 viruses (Nederland-1, Nederland-2, London-1, London-3, D48 (Paris), Geneva-1, and Roma-2) are practically indistinguishable from Paris-1. In these cases, the results of haemagglutination-inhibition and complement-fixation tests are entirely consistent.

TABLE V. CROSS-NEUTRALIZATION TESTS IN EGGS OF STRAINS OF INFLUENZA A VIRUS

Serum	50% neutralization titre against			
	Paris-1	Roma-2	Williams (England, 1947)	FM1
Anti-Paris-1	40	80	80	< 10
Anti-Roma-2	80	254		
Anti-Williams	< 10		320	
Anti-FM1	254			320
Antigenic ratio in relation to Paris-1	1 1	$\frac{1}{1.25}$	$< \frac{1}{4}$	$< \frac{1}{2.25}$

Neutralization test in eggs and in mice

Neutralization tests, on account of their variability and expense, have been performed only in a few cases, to confirm the results obtained by in vitro tests.

Egg test. Fourfold dilutions of inactivated sera in broth were mixed with equal volumes of diluted virus containing approximately 10^3LD50.

The mixtures were incubated at 4° C for one hour and 0.2-ml quantities were inoculated into the allantoic cavity of 10- to 11-day-old chick embryos ; at least four eggs were used for each serum dilution. Occurrence of virus multiplication was tested by putting up tests for haemagglutination by allantoic fluid after incubation for 72 hours at 35° C. 50% neutralization titres were calculated according to Reed & Muench.[16] Only the results from single experiments were analysed for antigenic ratios calculated in the same way as for haemagglutination-inhibition tests. The results of these tests are shown in table V, from which it can be concluded that Paris-1 is indistinguishable from Roma-2 and is related to, but distinct from, Williams (England, 1947) and FM1 (USA, 1947).

Mouse test. The homogeneity of viruses isolated in 1948-1949 contrasts with an antigenic diversity of types from one epidemic, as described in Britain in 1937 (Stuart-Harris et al. [20]) and by workers in the USA. The possibility arose that the difference was due to techniques : to the study of egg-adapted viruses by complement-fixation and haemagglutination-inhibition in 1948-49 as against neutralization tests in mice of mouse-adapted viruses in 1937. Accordingly, we studied four 1949 A-prime viruses by the technique used in 1937. After four passages in ferrets, convalescent ferret sera were obtained. Good adaptation to mice was successful after 8 (Ned.-1), 15 (Paris-1), and 22 (Roma-2) mouse passages. We carried out neutralization tests in mice using ferret sera prepared against four of the viruses (Paris-1, Ned.-1, Roma-2, London-1) and tested them against three of the viruses. The results revealed no evidence of antigenic diversity amongst these strains. A change in technical methods cannot account, therefore, for the present findings.

Identification of influenza B virus

Altogether, ten strains of B virus were received in this laboratory. They were responsible for localized outbreaks at times superimposed on a more extensive A epidemic. Influenza B strains isolated in recent years seem to be more or less different from Lee (Lazarus [10]). Some of them are appreciably inhibited by normal ferret and mouse sera so that their initial typing by haemagglutination-inhibition test is by no means easy. Hoyle's complement-fixation test[8] is type-specific but not strain-specific and should be the ideal method for distinguishing A from B viruses. The use of mouse-lung antigen, however, presents a difficulty, since mouse adaptation is not only a tedious procedure but may not be successful with certain B strains. In a study designed to compare the value of different complement-fixation antigens for influenza diagnostic studies, we were impressed by the superiority of chorio-allantoic membrane antigen in some respects over the mouse-lung antigen (unpublished data). Accordingly, we have adopted the complement-fixation test, using the chorio-allantoic membrane antigen and type-specific human serum for the initial typing of influenza B virus.

TABLE VI. INITIAL TYPING OF STRAINS OF INFLUENZA B VIRUS BY HAEMAGGLUTINATION-INHIBITION TEST AND TYPE-SPECIFIC COMPLEMENT-FIXATION TEST

Strains	Haemagglutination-inhibition by ferret sera [a]			Complement-fixation test with human sera [b]	
	Anti-PR8	Anti-FM1	Anti-Lee	Anti-A	Anti-B
Sweden-2/49	< 10	< 10	15	< 2	32
BUI (Sweden, 1949)	< 10	< 10	10	< 2	64
London-5/49	< 10	< 10	15	< 2	32
Czech.-1/49	< 10	< 10	30	< 2	64
Budapest-1/49	< 10	< 10	15	< 2	192
Budapest-2/49	< 10	< 10	20	< 2	64
Budapest-3/49	< 10	< 10	40	< 2	96
Warner-B (Australia)	< 10	< 10	< 10	< 2	64
Seattle-1 (USA, 1949)	< 10	< 10	< 10	< 2	48
Mil-B (Australia, 1945)	< 10	< 10	20	< 2	128
PR8	1920			192	2
FM1		480			
Lee			320	< 2	96

[a] Reciprocals of serum dilution which inhibited eight partial agglutinating doses of virus. Sera treated with cholera filtrate.

[b] Reciprocals of antigen dilution which fixed 2.5 m.h.d. complement with an excess (1/8 dilution) of human convalescent sera.

The technique of the test is generally similar to that described by Hoyle. For typing purposes, only an antigen titration with a fair excess of specific serum is necessary. The preparation of antigen, for the general outline of which we are indebted to Dr. von Magnus, is as follows :

10-day-old chick embryos were infected by the allantoic route with a 10^{-3} dilution of the seed virus. After 40 hours' incubation at 35° C, the whole chorio-allantoic membranes were removed, washed thoroughly in saline, and cut into small pieces. To each membrane, 1 ml of saline containing 0.05% sodium azide was added. After freezing and thawing three times, the material was centrifuged and the supernatant fluid constituted the antigen.

The results of examination of ten influenza B strains by the complement-fixation test (using human sera) and haemagglutination-inhibition test (using ferret sera treated with cholera filtrate) are shown in table VI. A comparison of the results obtained by the two tests soon demonstrates that correct typing could not be reached with confidence by the inhibition test, while unequivocal conclusions were obtained by the complement-fixation test. All ten strains are undoubtedly influenza B.

Discussion

The influenza epidemic of 1948-1949 in western Europe was, for many Continental countries, the most widespread for several years. Its lower incidence in Britain and Scandinavia may be associated with the fact that those countries, like America, had had an outbreak of influenza caused by A-prime virus only two years earlier, in 1947. The A-prime virus is not known to have been prevalent in the main part of western Europe that year except for one isolation in the Netherlands, where it did not, however, cause any epidemic in 1947. Failure of the western European epidemic to spread eastward may possibly be associated with a smaller volume of traffic between East and West. Influenza was apparently widespread in Poland, but there is no information available as to its type.

The origin of the 1948-1949 epidemic seems to have been in Sardinia, as described by Magrassi.[12] This was possibly related to an outbreak, said to have been due to influenza B, occurring in Sardinia, Sicily, and Calabria in the early summer of 1948 ; these are the three provinces in which the autumn influenza A outbreak occurred first. One is reminded of happenings in 1943 when, in Britain, Canada, and the USA, scattered outbreaks of influenza A occurred in the early summer (in Britain, following some influenza B). After a quiet period during the warmer months, influenza A flared up in September — an unusual season for north temperate regions.

Our studies have shown that, by either of two serological methods, recently isolated influenza A viruses can be placed in relationship with other strains obtained during the same season and with strains from other seasons. The two methods, haemagglutination-inhibition and complement-fixation, gave generally concordant results. Rather to our surprise, almost all the strains from western Europe, from Italy to Iceland, and one strain each from Canada and the USA, were antigenically very homogeneous. They fell into the A-prime type first recognized in Australia in 1946 (Cam strain) and occurring as the predominant type in America and Europe in 1947 (FM1 (USA), Barratt (England) strains). They were demonstrably rather different from some 1947 strains but were hardly distinguishable from others. One strain (Ocean Island 1/1948) was also of the A-prime type, but not very closely related to the European A-prime strains.

There are two views concerning the apparent country-to-country spread of influenza — one that it is a genuine phenomenon, the other that the spread is apparent only, and that an activation of endemic viruses takes place successively in different countries when conditions are favourable. This question has been discussed by Shope[18] and Andrewes.[1] The 1948-1949 experience suggests rather strongly that a true spread occurred, for, epidemiologically, there are definite indications of a generally northward spread from Italy, and the viruses obtained from all over western

Europe were of an antigenically homogeneous group. This may have been an unusual phenomenon ; at all events, it seems to have happened this time.

It seems likely that the relative homogeneity of 1949 A viruses may at least in part be due to the fact that, in the present study, the chances for laboratory variation of viruses were reduced to a minimum. Hirst [6, 7] reported similar antigenic homogeneity among viruses derived from widely separated localities in the USA and suggested that some of the antigenic heterogeneity described by others might be due to artefacts produced by laboratory manipulations. Undoubtedly, different antigenic varieties of influenza virus do occur in nature and may possibly occur even in the same outbreak ; but our experiences serve to emphasize that, as far as possible, the complicating factor of laboratory variation must be controlled in order that antigenic studies can most closely reflect the true behaviour of viruses as they occur in nature. Firstly, it is clear that different lines of standard viruses such as WS, PR8, and FM1 currently used in different laboratories may not be antigenically identical ; such antigenic differences are usually small but may occasionally be large and practically important, such as in the case of one variant of WS. It seems desirable that laboratories engaged in antigenic studies should agree to renew their standard viruses from a common stock least remote from primary isolation. Secondly, freshly isolated viruses in chick embryos should be freeze-dried as soon as possible. Further passages in eggs should be limited to a minimum and in no case should viruses passed in mice or ferrets be used for antigenic studies. The passage history of each strain should be indicated. Viruses used for production of serum and for preparation of antigen should be from the same, or the nearest possible, egg passage. Incidentally, these precautions will also reduce the chances of laboratory contamination, a risk always present in any laboratory maintaining multiple strains by prolonged serial passage.

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We wish to thank all those mentioned in the text and many others in many countries who have assisted in this study ; also our assistants, Mr. D. Busby and Mr. E. Owen.

ANNEX I. STRAINS OF INFLUENZA A ISOLATED IN 1948-1949

Country of origin	Designation	Place and date of isolation	Worker	Laboratory history when received [a]
Australia	FJS	Melbourne, February-March 1948	Anderson	
Canada	Ed-1	Edmonton, 1949	McClelland & Van Rooyen	
	Co-3	Cobourg, 1949	»	
	SC-1	St. Catherine, 1949	»	
	SSM-1	Sault Ste Marie, 1949	»	
	SJ-5	St. Johns, 1949	»	
	CP-1	Chorley Park, 1949	»	
	Eskimo-1/49	Victoria Island, Canadian Arctic, 1949	Van Rooyen	
	Eskimo-3/49	»	»	
	Eskimo-4/49	»	»	
	Eskimo-5/49	»	»	
France	A/Paris P.L. 1/49	Paris, 1949, isolated in London	Chu	Gargling
	Maurin	Paris, 1949	Lépine	E5
	Grillot	»	»	E5
	Chantal	»	»	E5
	Bacha	»	»	E5
	Copieux	»	»	E5
	Nourric	»	»	E5
	Fargeot	»	»	E5
	Wase	»	»	E5
	Chastaing	»	»	E5
	D48	Paris, 1948	Dujarric de la Rivière	
Germany	RK1	Berlin, April 1948	Henneberg	
Hungary	A/Budapest-4 49	Budapest, 1949	Farkas	
Iceland	A/Reykjavik-1/49	Reykjavik, 1949	Sigurdsson	E2
	A/Reykjavik-2/49	»	»	E2
	A/Reykjavik-3/49	»	»	E2
Italy	Napoli-4/49	Naples, 1949	Carlinfanti	
	A/Roma-1/49	Rome, 1949	Babudieri	F1 AM2 AL1
	A/Roma-2/49	»	»	AM3 AL1
	A/Roma-3/49	»	»	F1 AM1
	A/Roma-5/49	»	»	
	CIM	Cagliari, Sardinia, 1949	Magrassi	
	MUL	Sassari, Sardinia, April 1949	»	
	TUG	Sassari, Sardinia, 1949	»	
Netherlands	A/Nederland-1/49	Netherlands, 1949	Mulder	E2
	A/Nederland-2/49	»	»	E2
	A/Nederland-3/49	»	»	E21
	A/Nederland-4/49	»	»	E26
	A/Nederland-5/49	»	»	E15
	A'Nederland-6/49	»	»	E22
Ocean Island (South Pacific)	A/Ocean Island-1/48	Ocean Island, 1948	Isaacs	
Puerto Rico	Coamo-1	Puerto Rico, 1948	Perez	
Sweden	N782	Gotenburg, February 1949	Von Seipel	AM3 AL3
	N784	»	»	AM3 AL2
Switzerland	A/Geneva-1/49	Geneva, 1949	Werth	AM2 AL2
	A/Geneva-2/49	»	»	
Turkey	A/Ankara-1/49	Ankara, 1949	Payzin	M5
	A/Ankara-2/49	»	»	M5
	A/Ankara-3/49	»	»	M5
United Kingdom	A/London-1/49	London, 1949	Chu	Lung
	A/London-2/49	London, 1949 (visitor from Paris)	Dudgeon	M1 AM1 AL2
	A/London-3/49	Westminster School, London, 1949	»	AM2
	A/London-4/49	Chester Army Hospital, 1949	Chu	Gargling
	Rodway	London, 1949	Dudgeon	AM1 AL1
	Pearce	»	»	AM1 AL1
	Freeman	»	»	AM1 AL1
	Hendon	»	Gough	
	Oxford	Oxford, 1949	»	
	Burch	Northampton, 1949	Hoyle	

ANNEX 1. STRAINS OF INFLUENZA A ISOLATED IN 1948-1949 (Continued)

Country of origin	Designation	Place and date of isolation	Worker	Laboratory history when received [a]
United Kingdom	Mackinnon	Catterick, 1949	Stuart-Harris	E2
	Irvine	»	»	E1
	Ashmore	»	»	E1
	Bowers	Sheffield, 1949	»	E1
	Maude	»	»	E2
	Hill	»	»	E1
	Knowles	»	»	E2
	Worsop	»	»	E1
	Evans	»	»	E2
	Udall	»	»	E1
	Stone	»	»	E1
	Barker	»	»	E2
	Young	»	»	E2
	Brand	Edinburgh, 1949	Swain	
	Barclay	»	»	
	Duguid	»	»	
	Rennie	Glasgow, 1949	»	
	Shaw	Lanark, Scotland, 1949	»	
United States of America	Albany-1	New York, 1949	Gordon	
	Conn-1	New Haven, Conn., 1949	Curnen & Green	
	L4	Lawrenceville, N.J.	Siegel	
	Royer		Salk	
	Rosenfeld	Cleveland, Ohio, Spring, 1949	Feller	
	SF-1	San Francisco, spring, 1949	Weiss	
	Hood		Francis	AM1AL5

[a] AL = allantoic ; AM = amniotic ; E = egg ; F = ferret ; M = mouse. The number following indicates the number of passages in any particular host.

ANNEX 2. STRAINS OF INFLUENZA B ISOLATED IN 1948-1949

Country of origin	Designation	Place and date of isolation	Worker	Laboratory history when received [a]
Australia	Warner-B		Burnet	
Czechoslovakia	B/Czech.-1/49	Bratislava, 1949	Blaskovic	E4
Hungary	B/Budapest-1/49	Budapest, 1949	Farkas	
	B/Budapest-2/49	»	»	
	B/Budapest-3/49	»	»	
Netherlands	Roha	Leiden, March 1949	Mulder	E2
Sweden	B/Sweden-2/49	Stockholm, 1949	Gard et al.	AM5AL16
	BUI	Uppsala, 1949	Bjorkman	E9
United Kingdom	B/London-5/49	Haileybury, 1949	Gough	E3
United States of America	Seattle-1	Seattle, Washington, 1949	Lazarus	

[a] AL = allantoic ; AM = amniotic ; E = egg. The number following indicates the number of passages in any particular host.

SUMMARY

The necessity of studying influenza on a worldwide scale has become increasingly obvious during recent years and, as a result, WHO has established a World Influenza Centre (WIC) in the laboratories of the National Institute for Medical Research in London. The present article reviews the data collected by the WIC during the 1948-1949 epidemic and discusses comparative studies of the different virus strains.

The epidemic appears to have begun in Sardinia between the middle of September and the middle of October 1948. It passed to Sicily, then to the Italian mainland, Switzerland, and France. To the west, it reached North Spain ; to the east, it passed through Austria and Central Europe to Turkey. Coming from Belgium, it was reported in the Netherlands at the beginning of 1949, Great Britain at the same time, and Iceland at the end of January. Scandinavia was only very slightly affected. The virus strains received by the WIC from France, Great Britain, Iceland, Italy, the Netherlands, and Switzerland were all of the same type (1949 A-prime).

The first part of the article describes in detail the spread of the epidemic and gives an account of epidemiological observations and of results of serological tests carried out in various countries.

In the second part, the authors give an account of the serological studies made by the WIC on 20 of the 86 strains received during the epidemic. An effort was made to persuade investigators in all countries to adopt a uniform system for the designation of strains so as to make comparison easier. In the study of strains, the antigenic variation which viruses kept in a laboratory may undergo after passage in mice or chick embryos must be taken into consideration. This is particularly important when new strains must be compared with strains from previous epidemics, maintained in the laboratory.

The 20 strains showed considerable similarity in haemagglutination tests and

RÉSUMÉ

La nécessité s'étant fait sentir au cours des dernières années d'entreprendre sur le plan international l'étude de la grippe, l'OMS a établi un Centre Mondial de la Grippe (CMG) dans les laboratoires du National Institute for Medical Research, à Londres. Le présent article passe en revue les données réunies par le CMG au cours de l'épidémie de 1948-1949, et traite de l'étude comparée des diverses souches de virus.

L'épidémie semble avoir débuté en Sardaigne entre la mi-septembre et la mi-octobre 1948. Elle passa en Sicile, puis en Italie, en Suisse et en France ; à l'ouest, elle atteignit l'Espagne du nord ; à l'est, par l'Autriche et les pays de l'Europe centrale, elle gagna la Turquie. Venant de Belgique, elle fut signalée aux Pays-Bas au début de 1949, en Grande-Bretagne à la même époque, et en Islande à la fin de janvier. Elle n'eut guère d'importance en Scandinavie. Les souches de virus reçues au CMG, provenant d'Italie, de Suisse, de France, des Pays-Bas, de Grande-Bretagne et d'Islande, étaient toutes du même type (1949 A-prime).

La première partie de l'article décrit en détail le cheminement de l'épidémie. Les observations épidémiologiques et les résultats des examens sérologiques effectués dans divers pays y sont rapportés.

Dans la deuxième partie, les auteurs relatent les études sérologiques faites au CMG sur 20 des 86 souches reçues au cours de l'épidémie. Un effort a été fait pour engager les chercheurs de chaque pays à appliquer un système uniforme de désignation des souches, afin de faciliter la comparaison. Dans l'étude des souches, il faut tenir compte des modifications antigéniques que peuvent subir les virus entretenus en laboratoire, par suite du passage sur souris ou sur embryon de poulet. Ce fait est particulièrement important lorsqu'il s'agit de comparer des souches nouvelles aux souches provenant d'épidémies antérieures, entretenues au laboratoire.

Les 20 souches ont présenté des caractères très semblables en ce qui concerne

on examination by electron-microscopy. The authors describe the method used for carrying out a haemagglutination-inhibition test and discuss in detail the advantages of removing the non-specific inhibitor by crude filtrate of cholera vibrio cultures. In analysing the results of this test, the authors introduce the idea of an " antigenic ratio " between strains, making it possible to take into account the factor of reactivity or avidity, which has already been suggested by other authors.

When compared with old strains according to the methods described, the 1948-1949 A viruses proved to be of the A-prime type. None of them was related to the WS or PR8 strains. Antigenically, the viruses of the recent epidemic constitute a more homogeneous group than those studied until now. They are also so closely related to the strains of the 1946-1947 epidemic that it is difficult to distinguish with any certainty between the two groups. The results of other serological tests contributing to the differentiation of the virus strains—strain-specific complement-fixation, and neutralization in eggs and mice—are included.

Ten strains of virus B isolated during localized epidemics which occurred simultaneously with virus A epidemics were received at the laboratory. For the initial typing, the authors used the complement-fixation test with antigen prepared from chorio-allantoic membrane and type-specific human sera. This test gave better results than the haemagglutination-inhibition test.

In discussing the results of these experiments, the authors mention two views concerning the origin of the epidemic : one, that it spread from an initial focus ; the other, that it was due to the renewed virulence of endemic viruses in different countries. The antigenic homogeneity of the viruses which caused the epidemic in the various regions supports the first hypothesis. It seems likely that the

l'hémagglutination et l'aspect au microscope électronique. Les auteurs décrivent la méthode qu'ils ont appliquée pour effectuer l'épreuve d'inhibition de l'hémagglutination. Ils discutent en détail les avantages de l'élimination de l'inhibiteur non spécifique par l'extrait de culture de vibrions cholériques. Dans l'analyse des résultats de cette épreuve, ils introduisent la notion de « rapport antigénique » entre les diverses souches, qui permet de faire intervenir le facteur de « réactivité » ou d'« avidité » déjà signalé par certains auteurs.

Les virus A de 1948-1949, comparés aux souches anciennes selon les méthodes décrites, se sont révélés comme appartenant au type A-prime. Aucun d'eux n'est apparenté aux souches WS ou PR8. Les virus de la récente épidémie constituent, du point de vue antigénique, un groupe plus homogène que ceux qui ont été étudiés jusqu'à présent. Ils sont très proches aussi des souches de l'épidémie de 1946-1947, à tel point qu'il est difficile de différencier ces deux groupes avec certitude. Les auteurs donnent aussi les résultats d'autres épreuves sérologiques qui contribuent à la différenciation des souches de virus : l'épreuve de fixation du complément, spécifique de souches, et le test de neutralisation sur œuf et sur souris.

Dix souches de virus B, prélevées au cours d'épidémies localisées et superposées à celles dues au virus A, sont parvenues au laboratoire. Les auteurs ont eu recours, pour les déterminer, à l'épreuve de fixation du complément, et ont utilisé l'antigène préparé sur membrane chorio-allantoïdienne et le sérum humain spécifique de type. Cette épreuve a donné des résultats beaucoup plus sûrs que celle de l'inhibition de l'hémagglutination.

En discutant les résultats expérimentaux, les auteurs mentionnent les deux opinions émises au sujet de l'origine de l'épidémie : selon l'une, elle se serait propagée à partir d'un foyer initial ; selon l'autre, il s'agirait d'un regain de virulence de virus endémiques dans diverses régions. L'homogénéité antigénique du virus qui a causé l'épidémie dans les différents pays vient à l'appui de la première hypothèse. Il est probable que

homogeneity revealed by the serological investigation is due partly to the fact that the risks of variation in strains kept in laboratories have been reduced to a minimum in the studies carried out by the WIC. There is no doubt that different antigenic varieties of influenza virus do occur in nature. To estimate the true differences which exist between them, an effort must be made to control the factor of laboratory variation. For this purpose, freshly isolated viruses in chick embryos should be freeze-dried as soon as possible. Investigators engaged in serological research should agree to renew their standard viruses from a common stock as close as possible to the primary strain. The number of passages in eggs should be limited to a minimum, and in no case should a virus passed in mice or ferrets be used for antigenic studies.

l'homogénéité révélée par les examens sérologiques provient en partie du fait que les risques de variations dues à l'entretien des souches en laboratoire ont été réduits au minimum dans les études effectuées au CMG. Il est indubitable que diverses variétés antigéniques de virus grippal se rencontrent dans la nature. Pour avoir une idée précise des différences qui existent entre elles, il y a lieu d'éliminer dans toute la mesure du possible les variations survenant au cours des cultures en laboratoire. A cet effet, les virus fraîchement isolés sur embryon de poulet devraient être lyophilisés très tôt. Les chercheurs engagés dans les études sérologiques devraient renouveler leurs souches standard de virus, à partir d'une souche commune aussi proche que possible de la souche originelle. Le nombre de passages sur œuf devrait être réduit au minimum, et aucun virus passé sur souris ou furet ne devrait être employé pour les études de la structure antigénique.

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